Microinjection of mRNA into Somatic Cells

Maki Murata-Hori, *University of Massachusetts Medical School, Worcester, Massachusetts, USA*

Yu-Li Wang, *University of Massachusetts Medical School, Worcester, Massachusetts, USA*

Microinjection of messenger RNA (mRNA) represents a convenient, effective approach to probe protein functions during specific period of the cell cycle.

Advanced article

Article Contents

doi: 10.1038/npg.els.0002696

Introduction

Microinjection has been practised by cell biologists for some 100 years. It remains a highly effective technique for delivering membrane-impermeable molecules into living cells. Despite the development of alternative methods for expressing/loading molecules, recent advances in molecular biology and imaging have dramatically broadened the application and brought about a renaissance of this technique.

Alternative bulk-loading methods to microinjection include transfection, scrape loading, bead loading and electroporation. Although they are easy to perform and are able to load a large number of cells at once, they also need large quantities of materials and the loaded cells often require a prolonged period of recovery. In contrast, only microinjection allows the investigator to analyse individual cells before and soon after the treatment.

Expression or overexpression of a protein may be effected by the microinjection of complementary DNA (cDNA), mRNA or proteins. Injection of proteins achieves this goal instantly, but often requires tedious purification of the target protein. In contrast, cDNA, despite the ease of preparation, requires nuclear entry, transcription and translation before the target protein is expressed. The expression may be further delayed during certain stages of the cell cycle such as mitosis. Microinjection of mRNA combines the advantages of these approaches. Expression appears to take place rapidly after the microinjection even during mitosis, while the mRNA can be easily prepared from the corresponding cDNA by *in vitro* transcription using commercially available kits.

Outline of Methods

Preparation of mRNA

All the plastic-ware for mRNA must be autoclaved prior to use. Before *in vitro* transcription, cDNA must be incorporated into vectors of appropriate promoters such as T7. Capped and polyadenylated mRNA is synthesized following the manufacturer's instructions for the commercial kit. Transcribed mRNA is separated from unincorporated nucleotides by spin column gel filtration (Sigma, Cat. No. S-1295, St Louis, MO, USA), and is purified by phenol/chloroform extraction followed by chloroform extraction. mRNA is then precipitated by ethanol precipitation and resuspended in RNase-free deionized water (dH_2O) at a concentration of $\sim 2\,mg\,mL^{-1}$. The yield is estimated by ultraviolet (UV) absorbance at 260 nm using an extinction coefficient of 40 (Murata-Hori *et al.*, 2002). To facilitate the identification of injected cells, a fluorescent dextran (Molecular Probes, Eugene, OR, USA) may be included in the solution. In this case, the fluorescent dextran must be ultracentrifuged (e.g. in a Beckman TLX tabletop centrifuge, Palo Alto, CA, USA) at 100 000 *g* for 20 min before it is combined with the RNA solution.

Microinjection of mRNA into mitotic cells

Before loading the RNA solution into the needle, the RNA solution must be heat-treated for 1 min at 60°C (Wheatley *et al.*, 1997; Murata-Hori *et al.*, 2002), which causes the opening of secondary structures and facilitates the expression. The RNA solution is back-loaded into glass microinjection needles prepared from capillaries containing a glass fibre (Fredrick-Haer, Cat. No.#30-31-0, Brunswick, ME, USA), using a standard micropipette puller (David-Kopf Model 720, Tujunga, CA, USA). The capillaries were used as provided by the manufacturer without special treatment.

The mRNA solution is delivered into the perinuclear region of interphase cells or the cytoplasm of mitotic cells, with the needle under a steady air pressure of $\sim 1/3$ PSI (Wang, 1994). Microinjection of mitotic cells should be performed in a region far from the mitotic spindle because of its sensitivity to physical damage. Due to its spread morphology and resistance to microinjection, normal rat kidney (NRK) cells are used routinely for the study of mitosis (**Figure 1**).

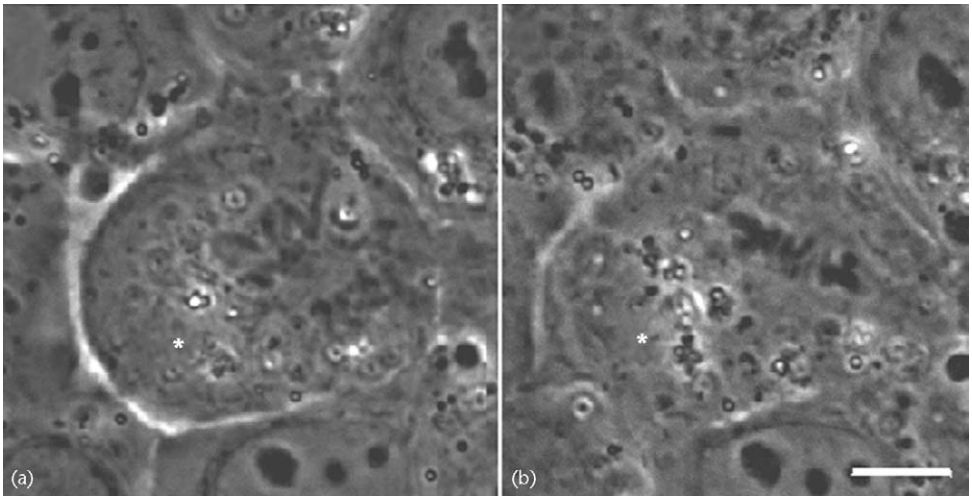

Figure 1 Mitotic normal rat kidney (NRK) cells. The cells are in prometaphase (a) and metaphase (b) In both cells, microinjection should be performed around the peripheral region of the cell (marked by asterisks) to avoid damaging the mitotic spindle by microinjection. Bar, 10 μm.

Observations and controls

To avoid artefacts, microinjected cells must be compared not only with uninjected cells but with cells injected with a control RNA of similar size, such as the mRNA for ovalbumin. Since microinjection inevitably causes transient physical damages, any short-term, transient (< 30 min) response must be confirmed with great caution. Depending on the experiments, injected cells may be subject to a battery of analyses such as time-lapse microscopy and immunofluorescence. To confirm the expression of the target protein, cDNA fusion constructs containing an epitope tag, such as FLAG or myc, may be used in conjunction with immunofluorescence using specific antibodies against the tag.

Example Applications

Probing functions of specific proteins in mitosis

Microinjection of mRNA may be used to express a dominant mutant form of a protein. For example, microinjection of mRNA for Δ90 cyclin B (Glotzer *et al*., 1991), a nondegradable mutant of cyclin B, into prometaphase NRK cells resulted in inhibition of the degradation of cyclin B at anaphase onset (Wheatley *et al*., 1997). Consistent with the effects of Δ90 cyclin B added to *Xenopus* egg extracts (Holloway *et al*., 1993), the injection had no effect on metaphase chromosomal alignment or anaphase chromosomal separation (Wheatley *et al*., 1997), but caused serious defects in the organization of the central spindle and in cytokinesis.

Understanding interaction between two proteins in mitotic cells

To test the effect of one protein on another, microinjection of mRNA may be combined with the transfection of cDNA for a green fluorescent protein (GFP)-tagged protein. Fluorescence microscopy of GFP would then reveal the effects caused by the expression of the protein coded by the injected mRNA. **Figure 2** shows one example. We microinjected mRNA for Δ90 cyclin B into an NRK cell expressing aurora B-GFP (a 'chromosomal passenger protein'), to assess if the distribution of aurora B-GFP is regulated by cyclin B degradation at anaphase onset. In contrast to control cells (A and B), where aurora B relocates from centromeres to the central spindle during anaphase, inhibition of cyclin degradation by Δ90 caused aurora B to remain associated at centromeres (C and D).

Summary and Perspective

Microinjection of mRNA is a useful technique for probing the functions of specific proteins. While the effects may not last as long as cDNA transfection, the prompt expression allows protein functions to be analysed in single living cells at defined stages of the cell cycle including mitosis. The approach may be readily combined with a number of imaging and molecular biological techniques. With the recent improvements of microinjection techniques, this approach is expected to provide new mechanistic insights into the functions of living cells.

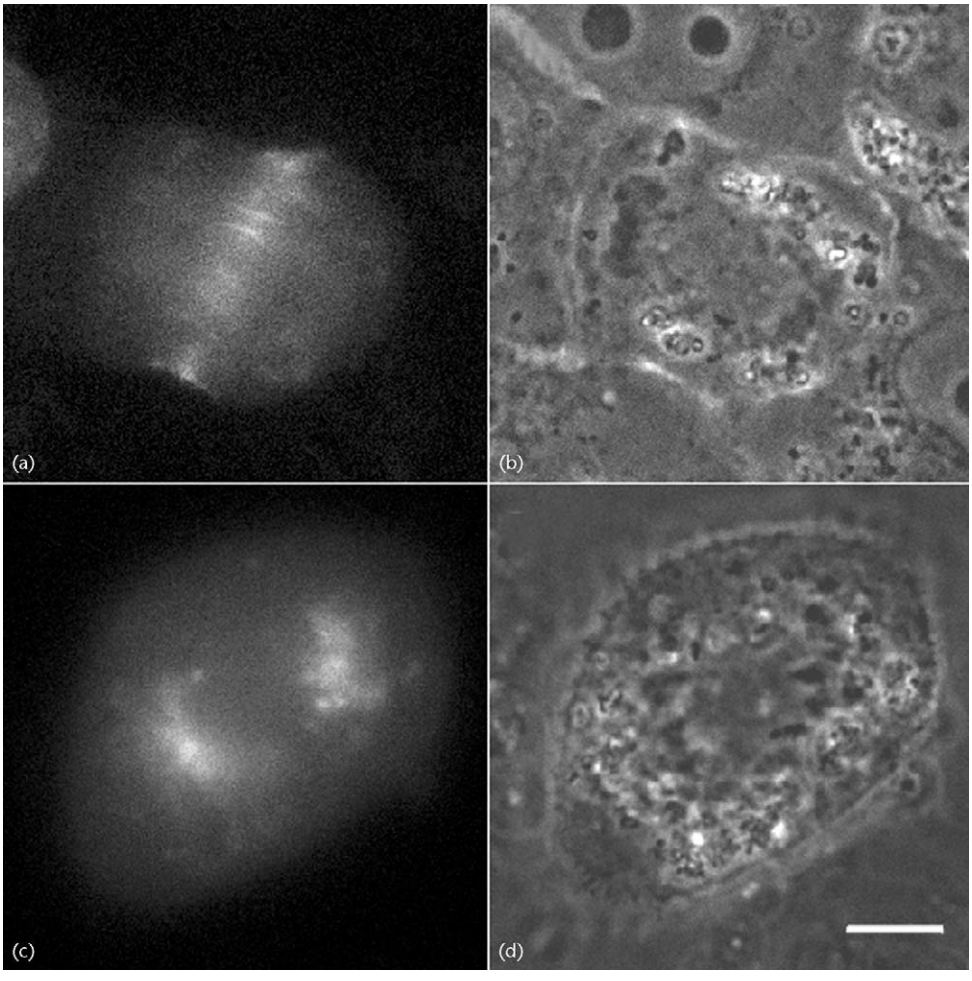

Figure 2 Microinjection of mRNA coding for a nondegradable mutant of cyclin B, Δ90, into an NRK cell expressing aurora B-GFP. (a and b) An uninjected NRK cell expressing aurora B-GFP. (c and d) An NRK cell injected with mRNA for cyclin B Δ90 during prometaphase and incubated until anaphase. Bar, 10 μm. In both cases, aurora B is localized at centromeres during prometaphase and metaphase (not shown). In the control cells (a and b), aurora B relocates from centromeres to the central spindle upon anaphase onset. However, in the cell microinjected with mRNA for cyclin B Δ90 (c and d), aurora B remains associated with centromeres during anaphase.

References

Glotzer M, Murray AW and Kirshner MW (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* **349**: 132–138.

Holloway SL, Glotzer M, King RW and Murray AW (1993) Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* **73**: 1393–1402.

Murata-Hori M, Tatsuka M and Wang YL (2002) Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. *Molecular Biology of the Cell* **13**: 1099–1108.

Wang YL (1994) Microinjection of proteins into somatic cells. In: Celis JE (ed.) *Handbook of Cell Biology* pp. 16–21. San Diego: Academic Press.

Wheatley SP, Hinchcliffe EH, Glotzer M, Hyman AA, Sluder G and Wang YL (1997) CDK1 inactivation regulates anaphase spindle dynamics and cytokinesis *in vivo*. *Journal of Cell Biology* **138**: 385–393.